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MOLECULAR EVIDENCE FOR PARAPHYLY IN *NYCTOMYS SUMICHRASTI*: SUPPORT FOR A NEW GENUS OF VESPER MICE?

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ABSTRACT

DNA sequences were obtained from the mitochondrial cytochrome-*b* gene of nine specimens of *Nyctomys sumichrasti* collected in Mexico and Central America. Phylogenetic analysis (Bayesian Inference) of these sequences document heretofore unrecognized patterns in genetic diversity among phylogroups that: 1) indicated substantial levels of genetic divergence among phylogroups; 2) resulted in paraphyly of taxa currently recognized as *N. sumichrasti*; and 3) argued for a re-assessment of the current taxonomy of *Nyctomys* and perhaps recognition of a new genus.

Key words: cytochrome-*b* gene, *Nyctomys sumichrasti*, phylogenetics, Sumichrast's vesper rat

INTRODUCTION

Nyctomys sumichrasti (Sumichrast's vesper rat; De Saussure 1860) is an arboreal rodent (Cricetidae, Tylomyinae, Musser and Carleton 2005) distributed from Jalisco, Mexico, to Panama (Hall 1981; Reid 2009). It inhabits evergreen lowlands and lower montane regions including cloud, secondary, riparian, and semi-deciduous forests (Hall 1981; Sánchez-Hernández et al. 1999; Cervantes et al. 2004; Hunt et al. 2004). Typically, *N. sumichrasti* prefers middle and upper level forest strata, rarely descending to the ground (Emmons 1997; Timm and LaVal 2000; Hunt et al. 2004). Given its arboreal to semi-arboreal behavior, specimens of *N. sumichrasti* are relatively rare in most museum collections and consequently a paucity of information is available concerning its ecology, genetic and morphologic variation, and systematics.

Nine subspecies of Sumichrast's vesper rat are recognized (*colimensis*, *costaricensis*, *decolorus*, *florencei*, *nitellinus*, *pallidulus*, *salvini*, *sumichrasti*, and *venustus*; Hall 1981), however the majority of information is restricted to the original description of each taxon and little has been reported concerning the taxonomy, geographic and genetic variation, or relationships among subspecies. Musser and Carleton (2005) noted that specimens in the United States National Museum could be separated into two groups based on variation in the carotid circulatory pattern (complete versus derived) and molar-root number (three versus four). Their groups corresponded to populations occurring north and west of the Isthmus of Tehuantepec (*pallidulus* and *sumichrasti*) versus those to the south and east (*costaricensis*, *decolorus*, *florencei*, *nitellinus*,

and *venustus*). No comments were made concerning *colimensis* and *salvini*. Karyotype data have been reported for two subspecies, with *N. s. colimensis* being polymorphic ($2n = 50-52$, $FN = 52-54$; Lee and Elder 1977; Haiduk et al. 1988) and *N. s. florencei* possessing a $2n = 50$ and $FN = 52$ karyotype (Bradley and Ensink 1987).

Herein we assess genetic variation among nine specimens representing five of the nine subspecies (Fig. 1) of *N. sumichrasti*. To accomplish this, DNA sequences from the mitochondrial cytochrome-*b* gene (*Cytb*) were obtained and then evaluated in a phylogenetic context.

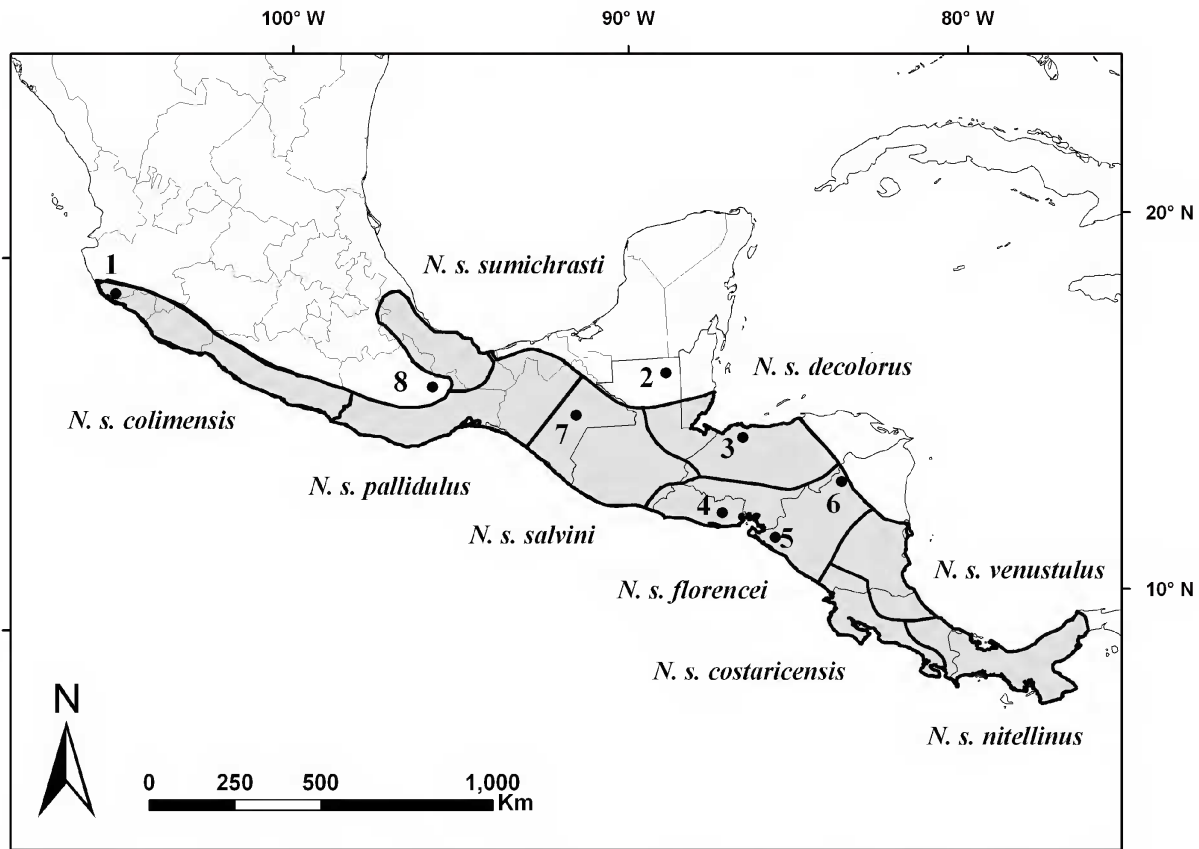


Figure 1. Map depicting collecting localities for samples examined in this study. Subspecies and distributions are recognized following Hall (1981). Numbers indicate localities as referred to in the Appendix.

METHODS

DNA sequences.—Mitochondrial DNA was isolated from approximately 0.1 g of frozen liver tissue using the Gentra Puregene Cell and Tissue Kit (Gentra Systems, Minneapolis, Minnesota) or from 0.1 g of liver preserved in 95% ethanol using the Qiagen DNeasy™ Tissue Kit (Qiagen Inc., Valencia, California). For most specimens, the entire *Cytb* gene (1,143 bp) was amplified by polymerase chain reaction (PCR, Saiki et al. 1988) using the following primers: MVZ05 (Smith

and Patton 1993) and PERO3' (Tiemann-Boege et al. 2000), or with primer pairs as follows: L14724 (Irwin et al. 1991) with CB3H (Palumbi 1996), and F1 (Whiting et al. 2003) with MVZ14 (Smith and Patton 1993). For other specimens, an approximately 400 bp fragment (at the 5' end) was amplified using primers MVZ05 and 400R (Peppers and Bradley 2000). The thermal profile for PCR reactions was as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denatur-

ation at 95°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 7 min. PCR products were purified using ExoSAP-IT (USB Products, Cleveland, Ohio). Primers used to cycle sequence the products consisted of MVZ05 and MVZ14 (Smith and Patton 1993), PERO3' (Tiemann-Boege et al. 2000), 870R (Peppers et al. 2002), F1 (Whiting et al. 2003), H15149, and L14841 (Irwin et al. 1991). Cycle sequencing reactions were purified using isopropanol cleanup protocols. Purified products were sequenced with an ABI 3100-Avant automated sequencer or with an ABI 377 automated sequencer using ABI Prism Big Dye version 3.1 terminator technology (Applied Biosystems, Foster City, California). Resulting sequences were subsequently assembled and proofed using Sequencher 4.9 software (Gene Codes, Ann Arbor, Michigan); chromatograms were examined to verify all base changes. The resulting DNA sequences were deposited in GenBank and the accession numbers are listed in the Appendix.

Data analyses.—Two approaches were used for data analysis. First, representatives of all tribes contained in the Neotominae (Bradley et al. 2004; Reeder and Bradley 2004, 2007; Musser and Carleton 2005; Reeder et al. 2006; Miller and Engstrom 2008) were included to test for monophyly of the subfamily Tylomyinae (Musser and Carleton 2005). The taxonomic sampling for this analysis included three members of the Neotomini (*Hodomys alleni*, *Neotoma mexicana*, and *Xenomys nelsoni*), one member of the Ochrotomyini (*Ochrotomys nutalli*), two members of the Baiomyini (*Baiomys taylori* and *Scotinomys teguina*), two members of the Reithrodontomyini (*Isthmomys pirrensis* and *Reithrodontomys fulvescens*), and eight members of the Peromyscini (*Habromys lepturus*,

Megadontomys thomasi, *Neotomodon alstoni*, *Onychomys arenicola*, *Osgoodomys banderanus*, *Peromyscus californicus*, *Peromyscus maniculatus*, and *Podomys floridanus*). For these taxa, sequences were obtained from GenBank (accession numbers are provided in the Appendix). Two members of the subfamily Sigmodontinae (*Oryzomys palustris* and *Sigmodon hispidus*) were used as outgroup taxa for this analysis.

In the second analysis, and based on results from the first analysis, the dataset was reduced to include only members of the Tylomyinae (*Nyctomys*, $n = 9$; *Otonyctomys*, $n = 1$; *Ototylomys*, $n = 1$; and *Tylomys*, $n = 1$). Given that *Ototylomys* and *Tylomys* were sister taxa in the first analysis, *Tylomys* was selected for the outgroup taxon. Sequences were deposited in GenBank (accession numbers are provided in the Appendix). Locality information for the specimens of *Nyctomys* is provided in the Appendix.

The GTR+I+G model was identified by the Akaike information criterion in MODELTEST (Posada and Crandall 1998) as the most appropriate model of DNA evolution for both datasets. A Bayesian model (MrBayes; Huelsenbeck and Ronquist 2001) was used to obtain a phylogenetic tree and to generate support values (clade probabilities). The GTR+I+G model parameters included: a site-specific gamma distribution, four Markov-chains, 10 million generations, and sample frequency = every 1,000th generation. After a visual inspection of likelihood scores, the first 1,000 trees were discarded and a consensus tree (50% majority rule) was constructed from the remaining trees. The Kimura two-parameter model of evolution (Kimura 1980) was used to obtain genetic distances and estimate divergence times between taxa.

RESULTS

The first Bayesian analysis produced a tree (not shown) depicting a monophyletic Tylomyinae (*Nyctomys*, *Otonyctomys*, *Ototylomys*, and *Tylomys*) and Neotominae (15 genera), with each clade being supported with probability values = 1.00. Based on these results, a second Bayesian analysis (Fig. 2) was conducted using *Tylomys* as the outgroup taxon (as explained in the Methods) in order to better assign

character polarity to the various sequences of *Nyctomys*. This analysis produced two major clades (I and II) and six minor clades (A–F) that were supported with probability values = 1.00. Clade I was comprised of two groups: *Otonyctomys hattii*, and seven of the nine samples of *Nyctomys*. Clade II consisted of the remaining two samples of *N. sumichrasti*.

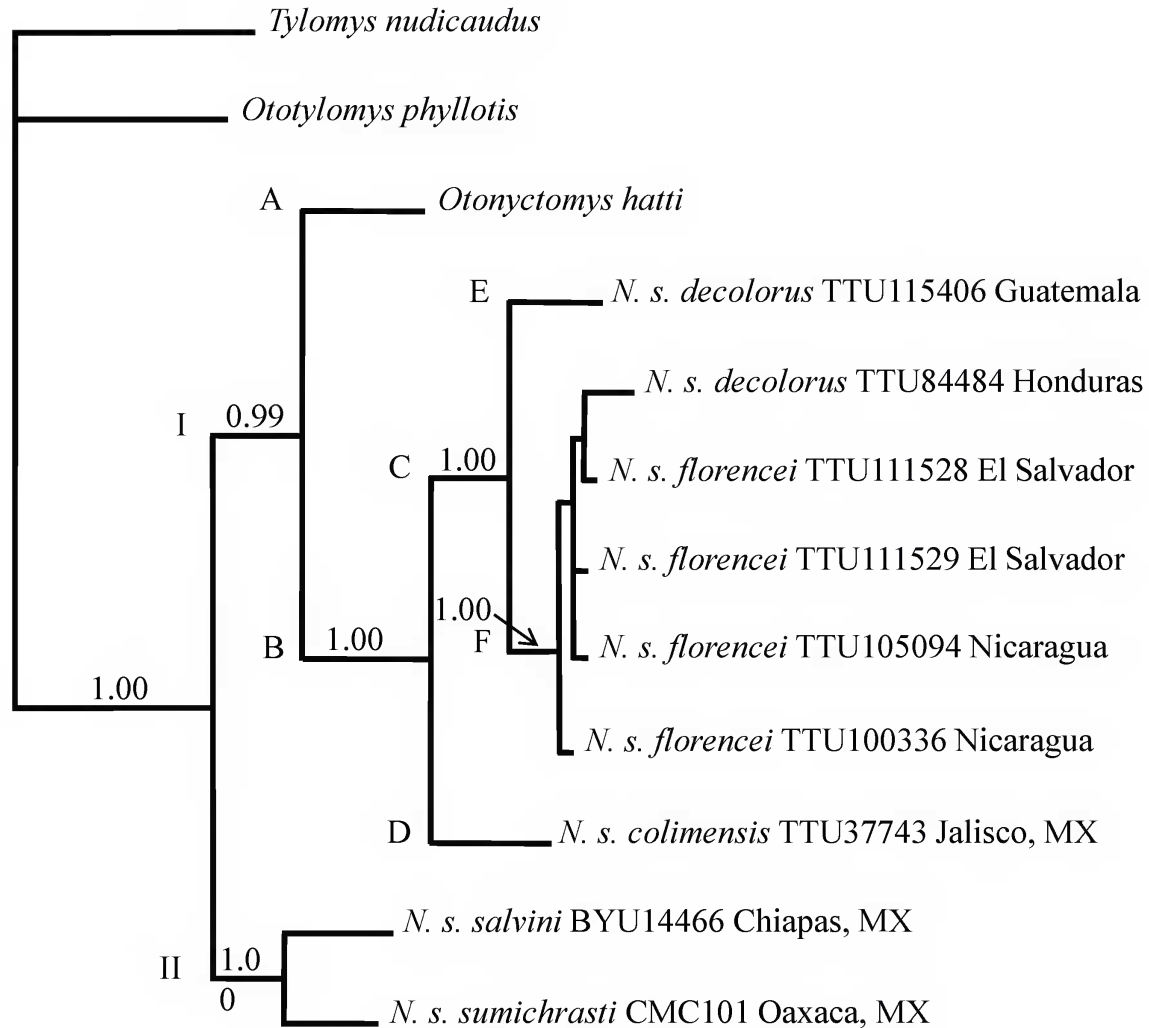


Figure 2. Phylogenetic tree obtained from the Bayesian analysis of mitochondrial cytochrome-*b* gene sequences. Values above branches indicate clade probability support values (only values ≥ 0.95 are shown).

Kimura two-parameter distance values (Kimura 1980) were obtained for comparisons among samples of *Nyctomys*, samples of *Nyctomys* versus *Otonyctomys*, and between other sister genera within the Tylomyinae and Neotominae (Table 1). Genetic distances obtained from within clade comparisons of samples of *Nyctomys* ranged from 1.5% (clade F) to 10.2% (clade II), whereas comparisons between clades containing samples of *Nyctomys* ranged from 8.4% (clades E and F) to 20.0%

(clades I and II). Using a sequence divergence estimate of 3.5% per million years for *Cytb* sequences (Arbogast and Slowinski 1998), divergence times and confidence intervals (obtained using the "POISSCI" function in MATLAB® version 6.5, The MathWorks, Inc. 2006) within clades ranged from 0.4 ± 0.34 mya (clade F) to 2.9 ± 0.58 mya (clade II) and between clades from 2.4 ± 0.54 mya (clades E and F) to 5.7 ± 0.86 mya (clades I and II) (Table 1).

Table 1. Genetic distances estimated using the Kimura 2-parameter model of evolution (Kimura 1980) for selected comparisons of *Nyctomys* and other members of the *Tylomyinae* and *Neotominae*.

Comparison	Genetic Distance	Time Since Divergence
Within Clades containing <i>Nyctomys</i>		
Clade I	9.3%	2.7 mya ± 0.56 mya
Clade II	10.2%	2.9 mya ± 0.58 mya
Clade B	6.5%	1.9 mya ± 0.49 mya
Clade C	3.8%	1.1 mya ± 0.41 mya
Clade F	1.5%	0.4 mya ± 0.34 mya
Between Clades containing <i>Nyctomys</i>		
Clades I and II	20.0%	5.7 mya ± 0.86 mya
Clades A and B	17.7%	5.1 mya ± 0.80 mya
Clades C and D	13.3%	3.8 mya ± 0.67 mya
Clades D and E	14.4%	4.1 mya ± 0.70 mya
Clades D and F	13.1%	3.7 mya ± 0.66 mya
Clades E and F	8.4%	2.4 mya ± 0.54 mya
Between other genera		
<i>Tylomys</i> and <i>Ototylomys</i>	21.3%	6.1 mya ± 0.90 mya
<i>Xenomys</i> and <i>Hodomys</i>	16.1%	4.6 mya ± 0.75 mya
<i>Baiomys</i> and <i>Scotinomys</i>	17.2%	4.9 mya ± 0.78 mya
<i>Reithrodontomys</i> and <i>Isthmomys</i>	18.0%	5.1 mya ± 0.80 mya

DISCUSSION

Phylogenetic analysis of *Cytb* sequences arranged samples of *N. sumichrasti* into two major clades (Fig. 2). The first clade (I) contained samples of *N. sumichrasti* from western Mexico and Central America together with the sample of *Otonyctomys hattii*. The second clade (II) contained the two samples of *N. sumichrasti* from southern Mexico. The inclusion of the sample of *O. hattii* with the nine samples of *N. sumichrasti* produced a paraphyletic arrangement and inferred a heretofore unrecognized set of relationships among the samples of *Nyctomys*. In addition, levels of genetic divergence, as reflected by the genetic distance data (Table 1), document an unusually high level of differentiation among clades. Together, these data suggest that a reassessment of the taxonomy of *Nyctomys* relative to *Otonyctomys* is necessary. Although the genealogy we recovered represents a gene tree and our sample size and geographic coverage is inadequate for a formal revision, several comments are appropriate.

Morphologically, specimens of *Nyctomys* are easily distinguishable from the monotypic *Otonyctomys*. *Otonyctomys* is smaller in the majority of cranial measurements, has much larger auditory bullae and a smaller maxillary toothrow (Anthony 1932; Genoways et al. 2005); therefore, placing *Otonyctomys* as a junior synonym of *Nyctomys* is inappropriate. On the other hand, the magnitude of genetic differentiation (Table 1) between the clades containing samples of *Nyctomys* and *Otonyctomys*, relative to other closely related genera of cricetid rodents, would support recognition of three genera: *Nyctomys* (members of clade II), *Otonyctomys*, and an unnamed genus (members of clade B). For example, levels of genetic divergence for comparisons of clades I and II (20.0%) and clades A and B (17.7%) were comparable for pair-wise values obtained from *Xenomys/Hodomys*, *Baiomys/Scotinomys*, *Reithrodontomys/Isthmomys*, and *Tylomys/Ototylomys* (ranged from 16.1% to 21.3%). Conversion of these distance

values into time since divergence estimates would place the origin of the unnamed genus in the late Miocene ($\sim 5.7 \pm 0.86$ mya), a date comparable to those of other Neotomine and Tylomyine genera (Table 1).

Regardless of generic relationships among clades representing *Nyctomys* and *Otonyctomys*, multiple species likely are included in what currently is recognized as *N. sumichrasti*. Depending on the species concept that is invoked (phylogenetic - Cracraft 1983; genetic - reviewed by Baker and Bradley 2006), it is possible to argue for the recognition of multiple species (perhaps as many as five) within the samples included in this study. Below, we follow the postulation of Baker and Bradley (2006) that genetic distance values $> 5\%$ (within the *Cytb* gene) exceed the average value for sister species of mammals, and as such, should be further evaluated in the context that they may represent separate species.

Clade I is comprised of three minor clades (D, E, and F) whose pair-wise distance values range from 8.4% to 14.4% (Table 1). The sample from Jalisco, Mexico (*N. s. colimensis*, clade D), occurs near sea level in tropical deciduous forests, is disjunct geographically from the remaining samples comprising clades E (Guatemala) and F (El Salvador, Honduras, and Nicaragua), and differs from them by 14.4% and 13.1%, respectively. Samples in clades E (*N. s. decolorus*) and F (*N. s. decolorus* and *N. s. florencei*) differ by 8.4%. This split between low elevation samples of *Nyctomys* is similar to the pattern recovered for *Alouatta* and *Mar-*

mosa mexicana (Baumgarten and Williamson 2007; Gutiérrez et al. 2010) and likely involved the Maya highlands as a barrier to dispersal between taxa located in the Yucatan Peninsula and those found further south. Clade II is comprised of samples from Chiapas, Mexico (*N. s. salvini*) and Oaxaca, Mexico (*N. s. sumichrasti*), that differ by 10.2%. Both samples are from cloud forests, separated by the Isthmus of Tehuantepec. The Isthmus has been recognized as an effective vicariant barrier for other mid- to high-elevation rodent taxa (Sullivan et al. 1997, 2000; Carleton et al. 2002; Edwards and Bradley 2002; Arellano et al. 2005; León-Paniagua 2007; Rogers et al. 2007).

Given the complex geography of southern Mexico and Central America (see Almendra and Rogers 2012 for a recent summary), it is possible that multiple lowland and highland forms exist, especially given the influence of the many isolated mountain ranges. Also, given the history of the Isthmus of Tehuantepec that included climatic changes coupled with marine incursions (Beard et al. 1982; Toledo 1982), it is possible that multiple invasions (from north to south, or the reciprocal) have taken place. Resolving these issues will require thorough geographic sampling and should include the addition of samples representing a more thorough geographic coverage. In addition, inclusion of unrepresented subspecies, ideally material from type localities, is essential for determining the priority of available names in cases where taxa should be elevated to species.

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Editor for this manuscript was Robert J. Baker.

APPENDIX

Specimens from which the cytochrome-*b* gene was sequenced are listed below with: locality information, museum voucher numbers, collection or tissue number, and GenBank accession numbers. The remaining sequences were obtained from GenBank (sequences generated in previous studies) and are listed by GenBank accession number.

Nyctomys sumichrasti colimensis.—MEXICO: Jalisco; 6 km SE Chamela, UNAM Estación de Biología (Locality 1, TTU37743, TK19590, JQ183066).

Nyctomys sumichrasti decolorus.—GUATEMALA: Petén; San Jose; Límite Oeste Biotopo San Miguel La Palotada-El Zotz, 17.17241°N, 89.88585°W, elev = 215 m (Locality 3, TTU115406, TK169284, JN851816). HONDURAS: Atlantida; Lancetilla Botanical Garden (Locality 4, TTU84484, TK101827, AF195801).

Nyctomys sumichrasti florencei.—EL SALVAOR: La Paz; about 3 mi NW San Luis Talpa (Locality 5, TTU111528, TK34799, JQ183063; TTU111529, TK34801, JQ183064). NICARAGUA: Chinandega; Chichigalpa, Belle Vista (Locality 6, TTU105094, TK113517, JQ183061); Atlantico Norte; Siuna, El Balsamo (Locality 7, TTU100336, TK121424, JQ183062).

Nyctomys sumichrasti salvini.—MEXICO: Chiapas; Municipio Chamela, Cerro Tzontehuitz, 13 km NE San Cristóbal de las Casas, 2,880 m (Locality 8, BYU14466, DSR4226, JQ183065).

Nyctomys sumichrasti sumichrasti.—MEXICO: Oaxaca; Distrito Ixtlán, 28 km SW (by road) La Esperanza, 17°35'08"N, 96°30'41"W, 2,950 m (Locality 9, CMC101, DSR5788, JQ183067).

Reference samples obtained from GenBank:

Neotominae.—*Baiomys taylori* (AF548469), *Scotinomys teguina* (JN851815), *Hodomys alleni* (AF186801), *Xenomys nelsoni* (AF307838), *Neotoma mexicana* (AF294345), *Ochrotomys nutalli* (AY195798), *Onychomys arenicola* (AY195793), *Reithrodontomys fulvescens* (AF176257), *Isthmomys pirrensis* (FJ214681), *Habromys ixtlani* (DQ973099), *Neotomodon alstoni* (AY195796), *Megadontomys thomasi* (AY195795), *Podomys floridanus* (EF989977), *Osgoodomys banderanus* (DQ000473), *Peromyscus californicus* (AF155393), and *Peromyscus maniculatus* (AY322503).

Tylomyinae.—*Otonyctomys hattii* (JQ183060), *Tylomys nudicaudus* (AF307839), and *Ototylomys phyllotis* (AY009789).

Sigmodontinae.—*Oryzomys palustris* (DQ185382) and *Sigmodon hispidus* (AF155420).